OCCURRENCE OF THE PROTOZOANS, LANKESTERELLA HYLAE AND HAEMOGREGARINA SP., IN THE BLOOD OF THE GREEN TREE FROG, LITORIA CAERULEA

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Of 62 green tree frogs, Litoria caerulea, collected in Queensland, 20 (32.25%) were found infected with sporozoites of Lankesterella hylae in their blood and a further 2 (3%) had gamerocytes of Haemogregarina sp. A single specimen from the Darwin district, Northern Territory, was negative for both parasites. A total of 594 specimens of 60 species of native anurans and 267 specimens of the introduced cane toad, Bufo marinus, proved negative for intraerythrocytic protozoans. It is suggested that there is a strict host specificity for these haemoprotozoans.

Lankesterella hylae, Haemogregarina sp., Litoria caerulea, haemoprotozoons, Hylidae, Australia.

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Lankesterella hylae is the only intraerythrocytic protozoan described from Australian anurans. Cleland and Johnston (1910) described it from the red blood cells of three out of five Litoria caerulea from Sydney. They reported the parasite was 9-11 by 1.6-3 μ m, crescentic with a band-like and reticular nucleus, which is often nearer one end than the other, and with a bulge containing a vacuole near the middle of the body. The parasite took various positions in the erythrocyte but did not distort or enlarge it, although the nucleus of the red blood cell was more or less displaced. They also noted double and triple infections and that the crescentic appearance was lost in free forms. The organism was again recorded in one out of three L. caerulea from Eidsvold, Queensland, by Cleland (1914) and from Brisbane as 'very common' by Johnston (1916). Mackerras and Mackerras (1961) found it in one out of ten specimens from Brisbane and six out of thirteen from Sydney. They reported measurements of 9-11 by 2-3 μ m for the most abundant forms and 6-8 by 2 μ m for the small forms. They observed numerous free parasites in organ smears from the heavily infected frogs, and exoerythrocytic stages in the liver and spleen. These forms occurred in small macrophages and they 'were oval crescentic, or sausage-shaped, with band-like reticular nuclei, and with 1 or 2 vacuoles'. Stehbens (1966a) found it in the erythrocytes of twelve out of thirteen L. caerulea from Gin-Gin, Queensland. The sporozoites (7-11 by 1-3 µm) were slender but in a number, one end was slightly less blunt than the

other, the nucleus was usually near the centre of the parasite and sometimes nearer the blunt end. At each end of the nucleus, he observed colourless vacuoles. On the concave surface of the intraery-throcytic forms he often noticed a 'dome-like swelling at or near the level of the nucleus' but could not determine its nature. He also made some observations from wet blood smears and on liver and spleen smears. The ultrastructure of L. hylae was studied by Stehbens (1966b).

In contrast, numerous haemoprotozoans have been reported from Bufo marinus. This species of toad is of South American origin and was introduced into Oueensland from Hawaii in 1935 in the hope that it would erradicate two species of devastating cane beetles (Mungomery, 1935, 1936). It is now widespread (Covacevich and Archer, 1975; Freeland, 1987). Hamerton (1932) reported Lankesterella associated with microfilaria in the blood of captive Bufo marinus in the collection of the Zoological Society of London. In 1934, he reported Lankesterella as a single infection and Lankesterella associated with 'Haemogregarines' in the blood of the same species. In South America, several species of Haemogregarina have been described in Bufo marinus. A complete list of the records for this toad is given in Table 1. However, none of the reported haemoprotozoans have been detected in Australia.

There is no record of the genus Haemogregarina in Australian anurans, but, in New Guinea, Ewers (1968) recorded 'hemogregarines' (= Haemogregarina Danilewsky, 1885, and Hepatozoon Miller, 1908) in 13 out of 20 Litoria infrafrenata, 7 out of 46 Platymantis papuensis and 6 out of 21 Rana papuensis. However, Walton (1964) did not include species of the genus Hepatozoon in his host-parasite list and, according to Manwell (1977) Hepatozoon occurs in mammals, birds and 'a number of cold-blooded vertebrates'.

MATERIALS AND METHODS

Frogs and toads were captured by hand at night using a spot light. Collections were made in 99 localities in all six States of mainland Australia between 1983 and 1985. Exact descriptions of the localities with longitude, latitude, and Grid Map Index, are in Delvinquier (1987). Within two to

TABLE 1. Intraerythrocytic protozoans reported in Bufo marinus in South America.

PARASITE	LOCALITY AUTHORITY		
Haentogregarina aguai Phisalix, 1930	Brazil	Phisalix 1930 ¹	
Haemogregarina bufomarinus Niño, 1926	Argentina	Niño 1926	
Haemogregarina cayennensis	Guiana	Lėger 1918a	
Léger, 1918	Brazil	Phisalix 1930	
Haemogregarinu darlingi	Guiana	Léger 1918b	
Léger, 1918	Brazil Venezuela Venezuela	Phisalix 1930 Scorza et al. 1956 see Díaz-Ungría 1960	
Haemogregarina legeri Scorza, Dagert and Arocha, 1956	Venezuela	Scorza et al. 1956	
	Venezuela	see Díaz-Ungría 1960	
Haemogregarina spp.	Panama (U.K.) ? Guiana (U.K.) Brazil	Darling 1912 ² Plimmer 1912a, 1912b Franca 1911 ³ Léger 1918b Hamerton 1933, 1934 de Figueiredo and Simões Barbosa 1943 Nickerson and Ayala 1982	
Lankesterella sp.	(U.K.)	Hamerton 1932, 1934	
Kuryolysus aguai Scorza, Dagert and Arocha, 1956	Venezuela Venezuela	Scorza et al. 1956 see Díaz-Ungría 1960	
Schellackia balli Le Bail and Landau, 1974	Guiana	Le Bail and Landau 1974	
Ductylosoma ranarum (Kruse, 1890)	Costa Rica	Ruiz 1959	
Dactylosoma sp.	Guiana	Le Bail and Landau 1974	
Cytamoeba bacterifera Labbé, 1894	Peru	Lehmann 1966	

¹ Scorza et al. (1956) believed this was Karyolysus aquai [sic] because of 'its agamic cycle is carried through in endothelial cells'. See also entry for Karyolysus aguai.

² Léger (1918b) reobserved it in B. marinus in Guiana and renamed it Haemogregarina darlingi.

³ According to Walton (1964), but he may have been misquoting the author or the date because I could not find the reference in *Zoological Record* or 'The Index-Catalogue of Medical and Veterinary Zoology'.

three days of their capture, each specimen was dissected after being anaesthetized with chloroform. Thin blood smears were fixed with methanol and stained with Giemsa 10% in sodium-potassium phosphate buffer at pH 7.0. In all, 924 specimens of 62 species in 5 families of anurans were examined for the presence of intraerythrocytic protozoans.

The numbers of frogs and toads dissected are as follows Inomenclature follows Cogger et al. (1983) and Czechura et al. (1987)]: BUFONIDAE: Bufo marinus, 267; HYLIDAE: Cyclorana brevipes, 1; C. novaehollandiae, 6; Litoria alboguttata, 1; L. caerulea, 63; L. chloris, 14; L. cyclorhyncha, 5; L. dahlii, 10; L. dentata, 5; L. ewingii, 3; L. fallax, 72; L. gracilenta, 5; L. inermis, 26; L. infrafrenata, 1; L. latopalmata, 20; L. lesueuri, 28; L. moorei, 1; L. nannotis, 2; L. nasuta, 36; L. nigrofrenata, 1; L. nyakalensis, 5; L. pallida, 21; L. pearsoniana, 3; L. peronii, 26; L. raniformis, 1; L. revelata, 2; L. rheocola, 11; L. rothli, 32; L. rubella, 26; L. serrata, 2; L. tornieri, 15; L. tyleri, 2; L. verreauxii, 1; Nyctimystes dayi, 10; MYOB-ATRACHIDAE: Adeloius brevis, 3; Assa darlingtoni, 4; Limnodynastes convexiusculus, 2; L. dorsalis, 3; L. dumerilli, 4; L. ornatus, 10; L. peronii, 21; L. salmini, 2; L. tasmaniensis, 13; L. terraereginae, 14; Mixophyes fasciolatus, 6; M. iteratus, 2; M. schevilli, 3; Neobatrachus centralis, 2; N. pelobatoides, 3; N. pictus, 4; Pseudophryne bibronii, 1; P. coriacea, 2; Ranidella bilingua, 9; R. insignifera, 1; R. parinsignifera, 20; R. signifera, 39; Taudactylus acutirostris, 3; T. rheophilus, 2; Uperoleia laevigata, 4; MICROHYLI-DAE: Cophixalus ornatus, 6; Sphenophryne robusta, 2; RANIDAE: Rana daemeli, 4.

Measurements are followed in brackets by the standard deviation of the sample (SDS), and number of specimens measured (N).

RESULTS

Lankesterella hylae and Haemogregarina sp. were the only intraerythrocytic protozoans that I found in Australian anurans. Both occurred in the red blood cells of Litoria caerulea. None of the other intraerythrocytic protozoans, Dactylosoma ranarum (Kruse, 1890) and Cytamoeba bacterifera (Labbé, 1894) were found.

Lankesterella hylae (Cleland and Johnston, 1910) (Figs 1,2,3)

MATERIAL EXAMINED
Commonwealth Institute of Health, University of

Sydney: Lankesterella hylae - Litoria caerulea, Sydney, 1909-10, 3 slides, Cleland and Johnston's collection [well stained specimens]; Lankesterella hylae - Litoria caerulea, 2 slides (well stained specimens on one slide; the other slide is labelled a 'scanty infection' but I did not find any specimens); Lankesterella hylae - Litoria caerulea, 2 slides, Mackerras and Mackerras's collection [well stained specimens]. Queensland Museum GL4863; United States National Museum 38856; British Museum (Natural History) 1987.1.19.1; Museum of Comparative Zoology (Harvard University) 8; all from Litoria caerulea, Kingaroy (151°51'E, 26°37'S; Grid Map Index: LR857550 9244-1), Queensland, April 1983.

DESCRIPTION

This protozoan was only observed in the erythrocytes of the common green tree frog, Litoria caerulea, in the localities listed in Table 2. Figure 1 gives the geographical distribution of Litoria caerulea based on Cogger (1983) with geographical records of the protozoan.

The sporozoite is a slender, crescentic form which measures on average (intracorpuscular form) 8.9 (SDS \pm 1.7) by 2.0 μ m (SDS \pm 0.7) (N = 33) (range; 5.8 to 14.6 by 0.7 to 3.6 μ m). The cytoplasm is pale blue when stained with Giemsa and contains in the middle an oval vacuole surrounded by masses of chromatin. This vacuole can be large enough to deform the shape of the sporozoite and create a bulge in its outline. The band-like nucleus is situated near one end of the parasite (see Figs 2,3). I did not find it in the leucocytes.

Infections were scanty, with a mean of only 2.3% erythrocytes infected (N = 4000 cells counted



Fig. 1. Geographical distribution of Litoria caerulea (stippled area) according to Cogger (1983) and records of Lankesterella hylae and Haemogregarina sp. Numbers refer to districts as in Table 2.

in smears stained with Giemsa). In the museum material, I found 2.0% erythrocytes infected in the three slides of Cleland and Johnston (N = 300), 1.7% in the well stained slide made by the anonymous collector (N = 100), and 6.6% in the two slides of Mackerras and Mackerras. Multiple infections, two sporozoites in one erythrocyte, were occasionally observed (see Fig. 3). A few free sporozoites were also seen. Erythrocytes were never deformed by the presence of the protozoan but the nucleus was slightly displaced.

COMMENTS

The form 1 observed in the erythrocytes of *Litoria caerulea* closely agrees with the description of the previous workers (Cleland and Johnston, 1910; Mackerras and Mackerras, 1961; Stehbens, 1966a).

Haemogregarina sp. of Litoria caerulea (Figs 1,4,5)

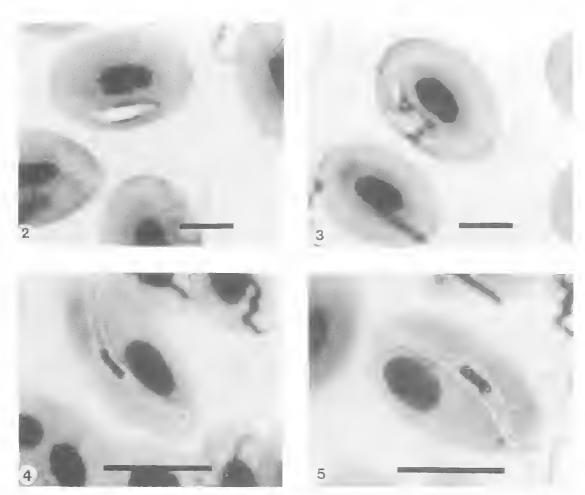
MATERIAL EXAMINED

Queensland Museum GL4864: Litoria caerulea, Bushy Creek, (145° 20'E, 16°36'S; Grid Map Index: CB223627 7964), Queensland, November 1983.

DESCRIPTION

I have observed this protozoan in only two specimens of *Litoria caerulea* from Bushy Creek, North Queensland (see Table 2). None of the 267 adult specimens of *Bufo marinus* were infected.

It has a narrow elongated body with rounded extremities. It is slightly bent around the nucleus of the erythrocyte. It measures on average 19.4



Figs 2-5. 2: Single infection of Lankesterella hylae in an erythrocyte of Litoria caerulea from Kingaroy. Scale bar: 10 μm. 3: Double infection of Lankesterella hylae in an erythrocyte of Litoria caerulea from Kingaroy. Scale bar: 10 μm. 4-5: Haemogregarina sp. in an erythrocyte of Litoria caerulea from Bushy Creek. Scale bar: 20 μm.

(SDS = 2.2) by 2.0 μ m (SDS = 0.4) (N = 23) (range: 16.0 to 23.3 by 1.5 to 2.9 μ m). The nucleus is situated in about the middle of the cell (see Figs 4 and 5). No bulge was observed. It typically deforms the erythrocyte it invades by elongating it. Only single infections were found in the erythrocytes. I observed the parasite in an average of 3% of the host erythrocytes (N = 100).

COMMENTS

I consider the form that I observed to be the gametocyte stage of a species of *Haemogregarina*. It has the features typical of the gametocytes of other species of that genus: intraerythrocytic, elongated, slender with rounded extremities, nucleus situated in the middle or near the narrower extremity.

TABLE 2. Occurrence and geographical distribution of Lankesterella hylae and Haemogregarina sp. in Litoria caerulea.

Localities	Collection dates	Number collected	Number infected
QUEENSLAND			
Brisbane district St Lucia Chapel Hill	Mar. 1983 Apr. 1983 Sep. 1983 Mar. 1983	1 1 2 1	0 0 0 0
2. Kingaroy district Kingaroy Kumbia Wooroolin	Apr. 1983 Jan. 1984 Jan. 1984	11 2 2	8 2 0
3. Gympie district Gunalda	Apr. 1983	5	5
4. Gayndah district Barambah Creek Gayndah Gayndah-Eidsvold Road	Jan. 1984 Jan. 1984 Jan. 1984	2 1 5	0 0 2
5. Bundaberg district Bundaberg	Jul. 1983	11	0
6. Rockhampton district Yeppoon	Aug. 1983	4	0
7. Mackay district Eungella-Mackay Road Marian	Nov. 1983 Nov. 1983	1 I	0
8. Townsville district Townsville	Nov. 1983	4	0
9. Atherton district Emerald Creek Mareeba Bushy Creek Herberton	Nov. 1983 Dec. 1984 Nov. 1983 Dec. 1984	2 1 4 I	1 I 2* 0
NORTHERN TERRITORY			
Darwin district Wildman River	Jun. 1985	1	0

^{*} Haemogregarina sp.

DISCUSSION

From my observations, as well as those of the previous workers, (Cleland and Johnston, 1910; Cleland, 1914; Johnston, 1916; Mackerras and Mackerras, 1961; Stehbens, 1966a). Lankesterella hylae occurs only in Litoria caerulea. Furthermore, this species of frog is so far the only Australian anuran to be found infected with a species of Haemogregarina. The absence of Lankesterella and of Haemogregarina in Bufo marinus is probably explained by a lack of infected specimens among the original stock (101 toads, see Mungomery, 1935), or to the absence of a suitable vector.

The highly restricted occurrence of Lankesterella and of Haemogregarina in Australian anurans calls for remark. Cleland and Johnston (1910), having noticed the common occurrence of Lankesterella hylae in the green tree frog Litorla caerulea and its absence in the blood of Litoria aurea, suggested that either the parasite was host specific, or that the intermediate host could not have access to L. aurea due to differences in the living habits of the two frog species. However, they regarded the latter hypothesis as unlikely. They also suggested that a leech was the intermediate host. Stehbens (1966a) thought that an insect such as a mosquito acted as the intermediate host. He had discovered intracellular parasites in the small intestine of the frog, within unidentified cells that he regarded as probably macrophages, or possibly enlarged and proliferated endothelial cells. He suggested that infection took place with ingestion by the green tree frog of an infected mosquito. The sporozoites released by the mosquito would enter the intestinal wall of the frog and develop into schizonts. Farmer (1980) considered that leeches were unlikely because 'the hosts are tree frogs' and instead suggested bloodsucking insects. On the other hand, Nöller (1913, 1920) demonstrated that a leech, Hemiclepsis marginata, passively transmits Lankesterella minima to Rana esculenta when the frog eats the leech. There is no evidence that a blood-sucking insect rather than a leech is the intermediate host in the life cycle of L. hylae.

L. caerulea has arboreal habits and is often found near houses where it is able to find water (for example in toilets and water tanks). I rarely found it near ponds, and when I did, it was after a warm rain shower at night during the breeding season. Other related arboreal species, such as the very common Litoria fallax, L. peronii, L. rubella, are sometimes found near houses in the same conditions. The former two species are also very

common in ponds and are calling most nights in summer whereas the green tree frog usually calls only after a warm shower, L. rubella was often found sitting, as was L. caerulea, on the road at night. From my own observations of the habits of species closely related to L. caerulea, I do not think that living habits of the green tree frog differ from these frogs in a way sufficient to explain the absence of the genus Lankesterella from other Australian anurans. I am therefore inclined to follow the first suggestion of Cleland and Johnston (1910) and believe that specificity of L. hylae is determined, but in an unknown way, by its ability to develop only in L. caerulea.

In the case of the genus Haemogregarina, the infection begins when an invertebrate blood-sucking vector bites the vertebrate host (Reichenow, 1910). Despite this difference, Haemogregarina sp. appears to be like Lankesterella hylae in being restricted to L. caerulea. I suggest that the specificity of Haemogregarina is determined in the same way as in Lankesterella.

The finding of 'hemogregarines" in Litoria infrafrenata from New Guinea by Ewers (1968) is interesting, L. infrafrenata is an hylid also found in North Oueensland 'in and around the remnants of rainforest on the eastern coastline of the Cape York (Tyler, 1976) and by its size and shape resembles very much Litoria caerulea. It may be that the Australian species also harbours some haemogregarines but I only captured one specimen of L. infrafrenata and it was negative. He also found 'hemogregarines' in the blood of tworanids, Rana papuensis and Platymantis papuensis. In Australia, there is only one ranid, Rana daemeli and the four specimens I collected were also negative. Records of the genera Lankesterella and Haemogregarina are mainly in ranids and in bufonids (see Walton, 1964).

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